Assessing the suitability of wetlands for the reintroduction of the Growling Grass Frog* *Litoria raniformis*

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ABSTRACT

The Growling Grass Frog Litoria raniformis has undergone population declines throughout its range over the past 20 years and is listed as a threatened species both in Victoria and nationally. The species was last recorded in 1979 in the area now occupied by the Portland Aluminium Smelter in south-western Victoria, but the reasons for its disappearance from the area are unclear. Approximately 50 wetlands remain in the buffer zone of the smelter and we investigated the suitability of 20 of these wetlands for the reintroduction of the species. We measured a set of habitat parameters at the smelter (Smelter sites) and at five wetlands in south-western Victoria that had recent records of Growling Grass Frogs (Extant sites). Discriminant function analysis identified a number of variables that discriminated between Extant and Smelter sites, particularly the extent of algae and the percentage of shoreline covered by bare ground, by short terrestrial vegetation and by other cover types. We recommend the planting of emergent aquatic vegetation and the encouragement of floating and submerged vegetation to increase habitat suitability for Growling Grass Frogs in the smelter wetlands.

Key words: Growling Grass Frog, habitat requirements, habitat suitability, Litoria raniformis, re-establishment, reintroduction, release site suitability.

Introduction

Declining amphibian populations have been documented around the world in both degraded and apparently pristine areas (e.g. Denton et al. 1997; Marsh and Pearman 1997; Pounds et al. 1997; Adams 1999; Houlahan et al. 2000; Knapp and Matthews 2000). Causes of these declines are often unclear and, in many cases, likely to be multiple and complex (see Ferraro and Burgin 1993; Adams 1999; Keisecker et al. 2001), including such factors as habitat degradation and loss, environmental pollution, predation by introduced fish, climate change, disease, and changes in hydrology and land use practices. This trend is also evident in Australia, where 23% of described anuran species are considered to be threatened with extinction, and a further 9% are near threatened or data deficient but of conservation concern (see Hero et al. 2006).

The Growling Grass Frog Litoria raniformis Kerferstein 1867, formerly found throughout much of south-eastern Australia, including Tasmania (Cogger 2000), was once considered abundant throughout its range (Draper 2001). Over the past 20 years, its range has contracted and numbers have declined dramatically (Rounsevell and Swain 1993; Tyler 1997). It has undergone local extinction in many areas, although apparently healthy populations persist in some parts of metropolitan Melbourne and regional Victoria (Tyler 1997; Robertson et al. 2002). The Growling Grass Frog is listed as nationally Vulnerable under the Australian Environment Protection and Biodiversity Conservation Act 1999 and in the Action Plan for Australian Frogs (Tyler 1997). The species is listed as threatened in Victoria under the Flora and Fauna Guarantee Act 1988 and is classified as Endangered on the state's Advisory List of Threatened Vertebrates (DSE 2003). It is also listed as Vulnerable in Tasmania (Tasmanian Threatened Species Protection Act 1992) and as Endangered in New South Wales (NSW Threatened Species Conservation Act 1999).

Growling Grass Frogs belong to the bell frog complex (L. aurea, L. castanea, L. cyclorhynchus, L. dahlii, L. moorea and L. raniformis; Thomson et al. 1996), half of which are in decline and one may now be extinct (Mahoney 1999). A number of causal factors have been implicated, including habitat loss, fragmentation and degradation (Tyler 1997; Robertson et al. 2002), increasing salinity (SAC 2000), predation of tadpoles and eggs by the introduced Mosquito Fish Gambusia holbrooki (Morgan and Buttemer 1996; Webb and Joss 1997; Anstis 2000; Pyke and White 2000), and poisoning by herbicides and pesticides (Tyler 1997). Increased levels of UV radiation are also a potential threat, as both adults and tadpoles have been observed basking in direct sunlight (van de Mortel and Buttemer 1996; Osborne et al. 1996; Tyler 1997; van de Mortel & Buttemer 1998; van de Mortel et al. 1998; Mahoney 1999). In addition, many declines in Victoria coincided with severe drought in the early 1980s (Tyler 1997; Robertson et al. 2002) and habitat fragmentation may have prevented remnant populations from recolonising suitable habitat.

* Referred to as the Southern Bell Frog in NSW

In 1980, the construction of an aluminium smelter commenced at Portland, on the west coast of Victoria, in an area comprised of freshwater swamps, native shrubland, heathland, grassland and exotic pasture (Alcoa and Kinhill 1980). In accordance with state legislation, an Environmental Impact Statement (EIS) was produced prior to the construction of the facility. Growling Grass Frogs were recorded during a fauna survey conducted as part of the EIS (Alcoa and Kinhill 1980) and listed as a species of 'special interest'. Three surveys of the area since then have failed to detect this species and it is believed to be absent from the smelter wetlands (Coulson et al. 2000). Although its disappearance apparently coincided with construction of the smelter, it is unclear whether the loss of the species is a direct consequence of the construction of the smelter or of other unrelated causes, particularly the 1982-93 drought, or some combination of the two.

In 1991, a management plan was developed by Portland Aluminium. The plan, 'The Smelter in the Park' (Brake et al. 1991), aimed to integrate the smelter and its surrounds into a multi-purpose park with an emphasis on conservation in some areas. The creation of 'The Smelter in the Park' has involved the implementation of waste minimisation and water management programs, and extensive, on-going habitat rehabilitation of the 200-ha buffer zone (Hill et al. 2000), incorporating both revegetation and pest control regimes. A series of reintroductions of locally extinct species is planned to further enhance biodiversity (Coulson et al. 2000).

Coulson et al. (2000) considered the Growling Grass Frog to be an ideal candidate for an experimental reintroduction into the park, as ample wetland habitat and abundant prey appeared to be present. However, a number of issues must be considered before reintroducing a species (see Sutherland 2000 for complete list). Perhaps the most important, and sometimes most elusive, is the identification and removal of the original cause of the decline (Sutherland 2000). These are often unclear, as in the case of the Growling Grass Frog, and environmental changes in the interim may make their identification prior to reintroduction impossible. Nevertheless, reintroductions, if properly designed, can provide information about current limiting factors; small-scale or short-term reintroductions with clear objectives and stringent monitoring can gauge the likelihood of success of the main project and determine whether alternative methods or strategies are necessary (Soderquist 1994). Other important components of any reintroduction are determining the habitat requirements of the species and whether suitable habitat exists at potential release sites (Griffith et al. 1989). For example, early attempts to reestablish the Natterjack Toad Bufo calamita in Britain were largely unsuccessful initially, but the success rate increased dramatically over time as knowledge of aquatic and terrestrial habitat requirements increased (Denton et al. 1997).

A number of parameters have been identified as important for the survival and reproduction of the Growling Grass Frog (Pyke 2002; Robertson et al. 2002). The species is generally found in water bodies with a variety of emergent, submerged and floating aquatic vegetation, suggesting that diverse vegetation structure is of primary importance (G. Marantelli, pers. comm. 2001, Amphibian Research Centre; R. Draper, pers. comm. 2001, Ballarat University). Although little is known about patterns of movement, foraging and diet, Growling Grass Frogs appear to be largely inactive between April and September, when hibernation may occur (Pyke 2002). Accordingly, both winter and diurnal shelter sites, such as rocks, cracks in the soil, fallen timber and thick vegetation or other debris, are considered important (Pyke 2002). While little information is available regarding specific water quality requirements, breeding occurs primarily in permanent, still waters (Hero et al. 1991; Cogger 2000) and slow-flowing areas of rivers and streams (Pyke 2002; Robertson et al. 2002), but has also been recorded in ephemeral water, such as ditches, artificial depressions and flood-irrigated areas (Pyke 2002).

We investigated the suitability of wetlands at the Portland Aluminium Smelter for the reintroduction of the Growling Grass Frog. After selecting a set of relevant habitat parameters, we compared values at the smelter wetlands with those at sites having recent records of Growling Grass Frogs in south-western Victoria. We sought to discover whether wetlands at the smelter differed significantly in the measured habitat parameters from those with recent records of the species and to determine which smelter wetlands were most similar to these extant Growling Grass Frog sites. We also evaluated the importance of the differing habitat parameters and made recommendations to increase the suitability of the smelter wetlands for Growling Grass Frogs.

Methods

Study sites were designated as either Smelter or Extant. Extant sites were those with records of Growling Grass Frogs in the past five years (Fig. 1a). Smelter sites were those within the buffer zone of Portland Aluminum (Fig. 1b). We chose these five Extant sites using records from the Atlas of Victorian Wildlife (NRE 2002) and our own observations. We selected only sites within 100 km of Portland Aluminium and 5 km of the coast to minimize variation in habitat type (i.e. substrate, climate and floristic species) between Extant and Smelter sites, because the smelter lies within 5 km of the coast. We chose Smelter sites from the two management zones where Growling Grass Frogs are likely to be reintroduced, Zones A and D, which had been designated for conservation and education purposes (Brake et al. 1991; Fig. 1b). We selected a total of 20 wetlands within these zones, treating connected wetlands as a single site.

We visited each site from June to October 2002 to record structural, vegetation and water quality parameters (Tables 1 and 2). Aquatic plant species were identified using Sainty and Jacobs (1994) and Romanowski (1998). We also searched for Mosquito Fish by dip-netting. We measured the length and width of smelter wetlands using a laser rangefinder, and calculated dimensions of Extant sites, which were larger, using a 1:25 000 map. We recorded the percentage of shoreline occupied by each dominant plant species and by non-vegetative material, and condensed these measurements into categories based on vegetation height (Table 3).

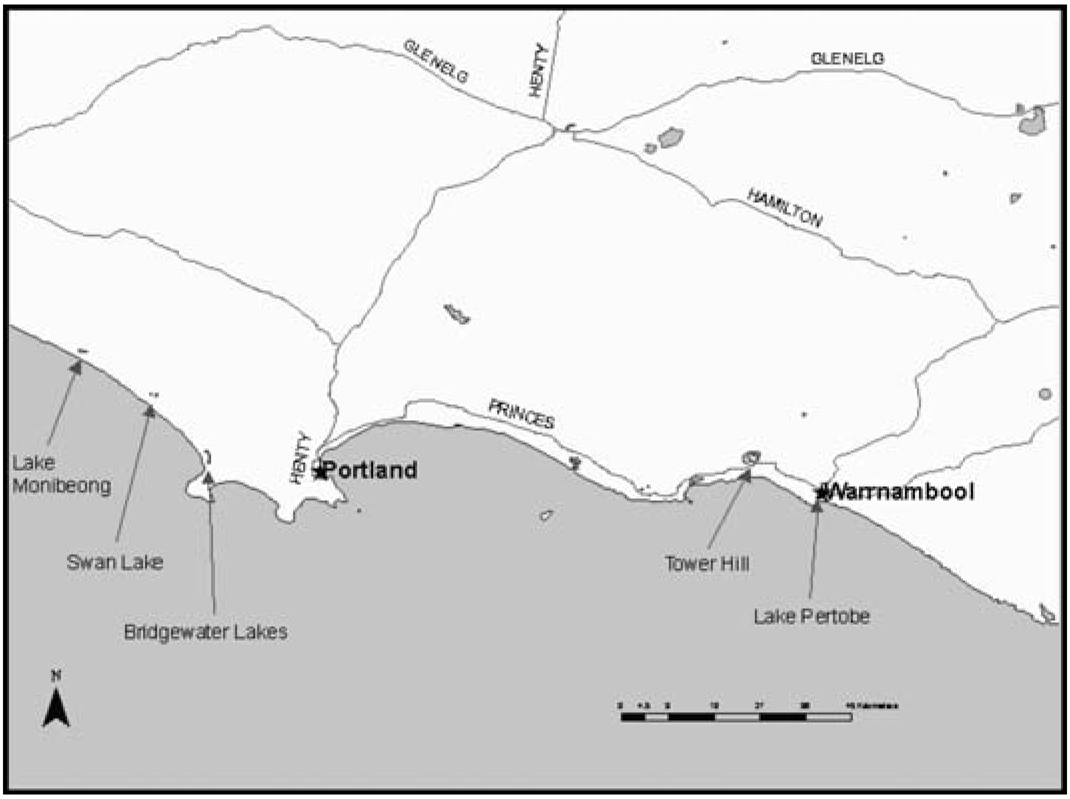

Figure 1a. Location of the five Extant sites assessed during this study (Lake Monibeong, Swan Lake, Bridgewater Lakes, Lake Pertobe and Tower Hill).

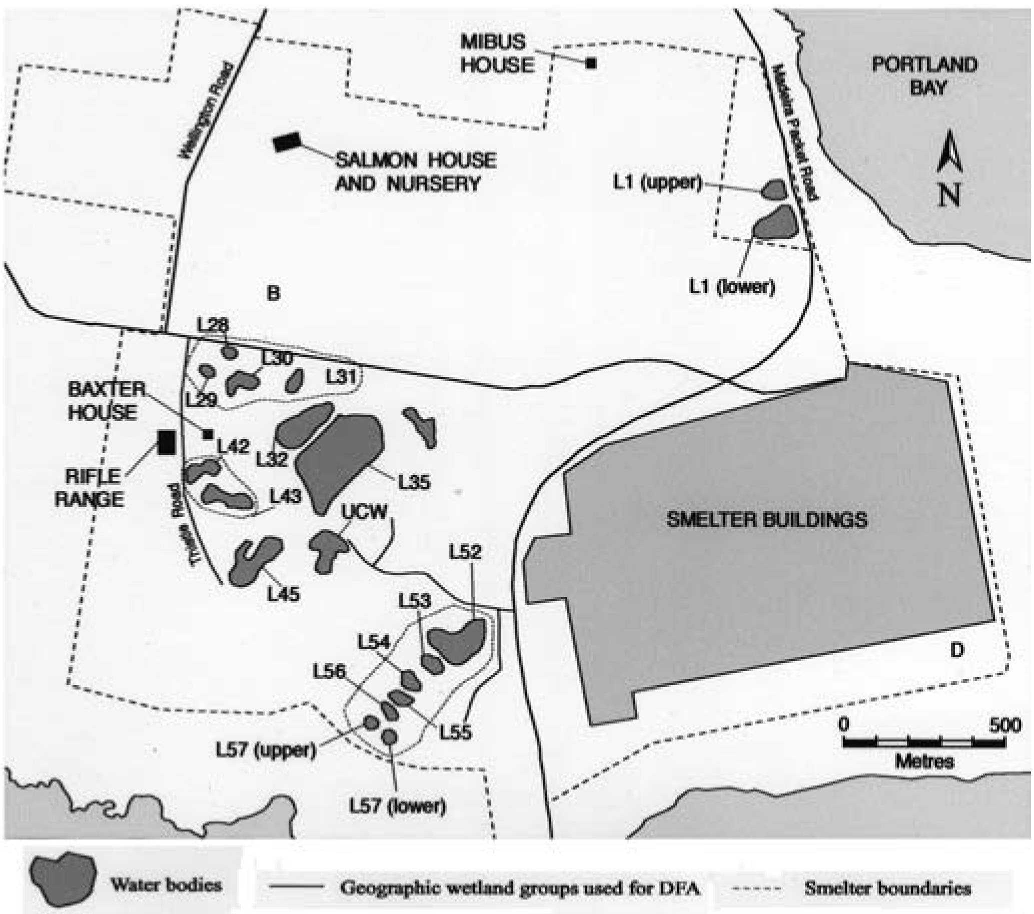

Figure 1b. Location of the Smelter sites assessed at Portland Aluminium (L1upper, L1lower, UCW, L28, L29, L30, L31, L32, L33, L35, L42, L43, L45, L52, L53, L54, L55, L56, L57upper, L57lower).

Table 1. Habitat parameters recorded at all study sites.

Structural parameters	Substrate type Length and width of water body Permanence of water body Presence of potential shelter sites and type Presence of mosquito fish ***Gambusia holbrooki*** Visible pollution Shade
Vegetation parameters	Aquatic plant species present in and within 5 m of the shoreline Presence and extent of algae and submerged, floating and emergent aquatic vegetation (see Table 2) % of shoreline that was bare ground % of shoreline with vegetative cover less than 5 cm high % of shoreline covered by emergent aquatic vegetation 5-30 cm high % of shoreline covered by emergent aquatic vegetation 30-60 cm high % of shoreline covered by emergent aquatic vegetation 60-100 cm % of shoreline covered by emergent aquatic vegetation more than 100 cm high % of shoreline covered by non-vegetative material or terrestrial vegetation
Water quality parameters	Dissolved oxygen (mg/L) Turbidity (NTU) Electrical conductivity (EC; μS/cm) pH

Table 2. Scores of vegetation extent. The absence of the vegetation type was scored as 0.

Vegetation type	Score	Description
Emergent aquatic vegetation (within water body)	1	Isolated emergent stems only
	2	Less than five sparse stands
	3	Greater than five sparse stands
	4	Less than five sparse-medium stands
	5	Greater than five sparse-medium stands
	6	Less than five sparse-medium-dense stands
	7	Greater than five sparse-medium-dense stands
Floating aquatic vegetation	1	Isolated floating stands only
	2	Numerous floating strands; lots of clear water
	3	High densities of floating strands; extensive areas of clear water
	4	Very high densities of floating strands; little clear water
Submerged aquatic vegetation	1	Occasional submerged plants only
	2	Numerous submerged plants; extensive bare substrate remaining
	3	Numerous submerged plants; little bare substrate remaining
	4	Unable to judge extent of submerged vegetation
Algae	1	Small isolated areas only
	2	Larger areas
	3	Extensive areas

Table 3. Cover types recorded at each site.

Cover category	Description
Bare	Bare dirt, sand or rock Primarily bare ground, occasional isolated emergent stems or grassy tufts
Ground cover only	Terrestrial plants less than 5 cm high, may contain bare patches Primarily terrestrial ground cover less than 5 cm high, isolated emergent stems or grassy tufts greater than 5 cm high
Other	Non-vegetative cover, including rocks, logs, branches, tyres, etc. Overhanging trees or shrubs, no aquatic vegetation present Grasses or other terrestrial species
Emergent vegetation less than 30 cm high	Mixed emergent aquatic species (no obvious dominant species) *Eleocharis acuta* *Baumea* spp. *Chorizandra* spp. *Schoenoplectus* spp.
Emergent vegetation 30-60 cm high	Mixed emergent aquatic species (no obvious dominant species) *Eleocharis acuta* *Schoenoplectus* spp. *Juncus* spp.
Emergent vegetation 60-100 cm high	Mixed emergent aquatic species (no obvious dominant species) *Juncus* spp. *Baumea* spp. *Chorizandra* spp. *Phragmites australis*
Emergent vegetation greater than 100 cm high	Mixed emergent aquatic species (no obvious dominant species) *Lepidosperma longitudinale* *Eleocharis sphacelata* *Juncus* spp. *Phragmites australis* *Typha* spp.

For larger water bodies, where recording cover types along the entire shoreline was impractical, we chose eight 50-m sections of shoreline by dividing each water body into four, roughly equal, sections on a 1:25 000 map and using the boundaries of these sections as reference points. We recorded the latitude and longitude of each reference point and later located them in the field using a Magellan GPS unit. We then took a random number of steps along the shoreline in both directions from each reference point to reach a starting point from which cover types were recorded for 50 m. This method of stratified random sampling ensured that spatial variation in cover types around the perimeter of the water body was considered.

We recorded all water quality measurements in the field over a 2-day period (15-16 October 2002) to minimize variation caused by rainfall and other climatic events. We measured dissolved oxygen, pH, temperature and salinity using a WTW Multi 340i Universal Pocket Meter and measured turbidity with a WP89 Turbidimeter, taking three measurements at each water body at a depth of approximately 20 cm to obtain a mean value for use in statistical analyses.

Results

We used monotonic, multi-dimensional scaling (MDS) to identify differences between Extant and Smelter sites, scaling all data so that the range for each variable was 0–100. MDS produced a two-dimensional configuration (Fig. 2). Tower Hill, an Extant site, was markedly different from all other sites, with the remaining Extant sites grouped together at the right-hand side of the configuration. Smelter sites were distributed over most of the configuration and there was some overlap between Smelter and Extant sites, suggesting that some Smelter sites were likely to be suitable for reintroduction of

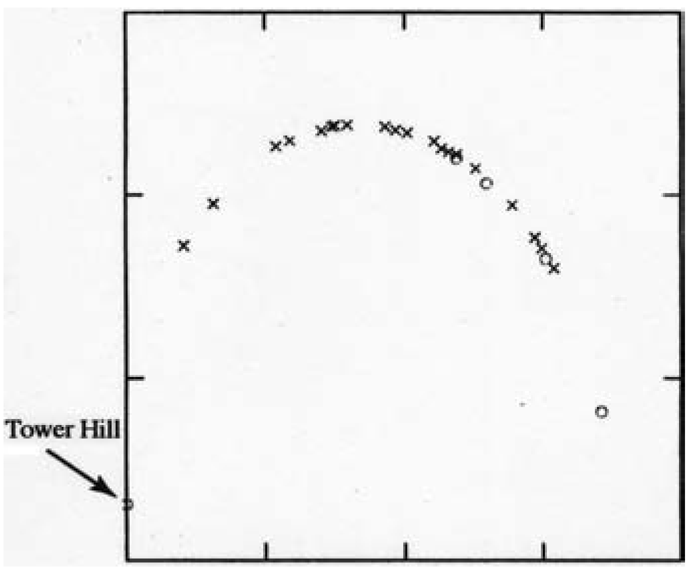

Figure 2. Two-dimensional configuration produced by MDS (Kruskal stress = 0.01157) showing relationships between sites; o denotes Extant sites, x denotes Smelter sites. All data were scaled so that the range for each variable was 0-100.

Growling Grass Frogs.

Extant sites were generally larger than Smelter sites (mean ± SE: Smelter sites = 9673 ± 3428 m2; Extant sites = 473,091 ± 181,602 m2). The substrate at all sites was sand, except for Tower Hill, which lay on basalt and basalt-derived soils. All Extant sites were classified as permanent and held water for the duration of this study, although large fluctuations in water levels were observed at some sites. Most Smelter sites were also considered permanent, although five wetlands (UCW, L28, L29, L33 and L43) filled only seasonally, and water levels at all sites rose during this study. While all Extant sites had some shade at the water's edge, only 14% of Smelter sites (L28, L30 and L31) had shaded edges and none were extensively shaded. No sites were obviously heavily polluted; visible pollution was limited to occasional rubbish and was observed at both Extant and Smelter sites. Potential shelter sites were present at all Extant sites, including rocks, fallen timber and branches, and dense undergrowth, and 81% of Smelter sites had similar shelter sites. Sites L28, L29, L33 and L57 were the only ones lacking obvious shelter nearby. We did not detect Mosquito Fish at any site.

We recorded a total of 20 taxa of submerged, floating and emergent vegetation (Table 4). Of these, 82% were found at both Extant and Smelter sites. Algae were present at all Extant sites and at 44% of Smelter sites. Floating vegetation was recorded at 80% of Extant sites and 86% of Smelter sites. Submerged vegetation was recorded at 80% of Extant sites and 76% of Smelter sites. Emergent vegetation was present at all sites. Water quality parameters varied considerably among both Smelter and Extant sites, although there was some overlap between the ranges observed for the two groups and values for Smelter sites often fell within the ranges observed at Extant sites. Smelter wetlands were generally more saline than Extant sites, with electrical conductivity (EC, measured in microSiemen/cm) ranging from 588-1656 μS/cm at Extant sites (excluding Tower Hill; 13 000 μS/cm) and from 991-5385 μS/cm at Smelter sites. Extant sites were less turbid, with turbidity ranging from 0.06-6.15 NTU, whereas turbidity at Smelter sites ranged from 1.41-98.05 NTU. All measurements of dissolved oxygen at Smelter sites (2.81-14.68 mg/L) fell within the range observed at Extant sites (9.49-16.15 mg/L) and pH ranges were also very similar, ranging from 7.2-10.3 at Extant sites and from 6.9-10.3 at Smelter wetlands. Water quality measurements for all sites are detailed in Table 5.

We used linear Discriminate Function Analysis (DFA) to determine whether sites could be separated into groups of sites that were potentially Suitable or Unsuitable for Growling Grass Frogs using the habitat parameters

Table 4. Aquatic vegetation species recorded at Smelter and Extant sites.

Species	Growth form	Extant sites	Smelter sites
Ferny Azolla *Azolla pinnata*	Floating	●	●
Twigrushes *Baumea* spp.	Emergent	●	●
Bristlerushes *Chorizandra* spp.	Emergent	●	●
Swamp Crassula *Crassula helmsii*	Submerged/emergent	●	
Flatsedges *Cyperus* spp.	Emergent	●	
Common Spike-rush *Eleocharis acuta*	Emergent	●	●
Sawsedges *Gahnia* spp.	Emergent	●	●
Floating clubrush *Isolepsis fluitans*	Submerged/floating	●	
Swordsedge *Lepidosperma longitudinale*	Emergent	●	●
Rushes *Juncus* spp.	Emergent	●	●
Watermilfoil *Myriophyllum* spp.	Submerged	●	●
Slender Knotweed *Persicaria deceipiens*	Emergent	●	●
Common Reed *Phragmites australis*	Emergent	●	●
River Buttercups *Ranunculus inundatus*	Submerged/emergent	●	●
Sharp Clubrush *Schoenoplectus pungens*	Emergent	●	●
Swampweed *Selliera radicans*	Submerged/emergent	●	●
Duckweeds *Spirodella* spp.	Floating	●	
Water Ribbon *Triglochin procerum*	Floating	●	●
Cumbungi *Typha* spp.	Emergent	●	●
Water Buttons *Cotula* spp.	Emergent	●	●

Table 5. Water quality measurements for all sites.

Site	Type	pH	Salinity (EC; µS/cm)	Turbidity (NTU)	Dissolved oxygen (mg/L)	Temperature (°C)
L1(upper)	*Smelter*	7.8	991	5.53	9.28	19.9
L1(lower)	*Smelter*	8.1	1114	1.49	7.57	18.5
UCW	*Smelter*	8.8	1826	5.57	8.64	19.9
L28	*Smelter*	7.4	1278	3.26	7.43	15.8
L29	*Smelter*	7.1	1525	2.70	3.62	15.3
L30	*Smelter*	7.7	3390	1.93	8.57	16.8
L31	*Smelter*	7.3	2725	1.41	9.45	20.0
L32	*Smelter*	8	1665	1.67	6.96	18.0
L33	*Smelter*	6.9	4550	1.51	10.79	18.3
L35	*Smelter*	9.3	3215	3.05	8.72	17.9
L42	*Smelter*	6.7	1069	3.25	2.81	13.4
L43	*Smelter*	7.4	2680	23.80	7.25	17.8
L45	*Smelter*	8	5385	11.49	8.57	16.5
L52	*Smelter*	10.3	2840	3.69	12.88	17.5
L53	*Smelter*	9.2	2555	16.20	11.34	17.9
L54	*Smelter*	9.2	2190	98.05	11.93	17.8
L55	*Smelter*	9.8	2110	38.60	11.99	19.1
L56	*Smelter*	9.7	2020	13.16	11.70	17.4
L57(upper)	*Smelter*	10.1	1765	9.02	14.68	17.7
L57(lower)	*Smelter*	10.2	1710	6.11	12.70	17.7
Bridgewater Lakes	*Extant*	8.9	1656	0.06	9.49	17.5
Swan Lake	*Extant*	7.2	588	0.74	9.62	16.5
Lake Monibeong	*Extant*	8.4	1033	1.02	10.64	15.7
Lake Pertobe	*Extant*	10.3	1437	3.47	10.30	18.6
Tower Hill	*Extant*	9.7	13000	203	16.15	22.4

measured. As DFA requires that the number of cases exceeds the number of variables (James and McCulloch 1990), we used Principal Components Analysis (PCA) to determine which of the 20 variables contributed most to variation among the 25 sites. We excluded the Tower Hill site from the PCA analysis; the MDS showed this site to be quite unlike all other sites, so its inclusion was likely to result in a misrepresentation of influential variables. PCA identified seven variables as contributing most to between-site variation (Table 6): percentage of bare shoreline (no vegetative or non-vegetative cover); extent of algae; percentage of shoreline with vegetative cover less than 5 cm high; extent of submerged vegetation; percentage of shoreline covered by emergent aquatic vegetation 5-30 cm high; percentage of shoreline covered by 'other' cover types (non-vegetative material or terrestrial vegetation); percentage of shoreline covered by emergent aquatic vegetation 30-60 cm high.

We used these seven variables with the highest PCA loadings in the DFA. DFA also requires similar sample sizes in each group of interest (James and McCulloch 1990; Quinn and Keough 2002), so we reduced the 20 Smelter sites to nine representative sites through a stratified randomized procedure: groups of geographically-close wetlands (see Fig. 1b) were identified and one wetland in each group was chosen at random. The resulting discriminate function correctly classified (jack-knifed classification, Table 7) 96% of sites as either Suitable (i.e. Extant) or Unsuitable (i.e. Smelter) (Wilks' lambda = 0.137, approx. $F = 5.401$, $df = 7, 6$, $P = 0.028$) (Table 6). Although we excluded Tower Hill from the PCA, it was included in the DFA and classified correctly as a Suitable site (Table 7). F-to-remove statistics showed that the extent of algae was the best discriminator between Suitable and Unsuitable sites, followed by the percentage

Table 6. Mean (range), PCA component loadings and F-to-remove statistics for variables used in DFA.

Variable	PCA loading[a]	F-to-remove[b]	Tolerance[c]	Smelter sites (n=21)	Extant sites (n=5)
Bare shoreline (%)*	0.790	0.93	0.60	31.39 (0-99.77)	14.48 (0-33.80)
Terrestrial vegetation less than 5 cm tall (%)*	0.777	0.97	0.74	14.38 (0-97)	3.82 (0-9.60)
Other cover types (%)*	0.675	4.25	0.40	7.81 (0-48.12)	25.75 (0-73.16)
Emergent aquatic vegetation 5-30 cm tall (%)	0.668	0.02	0.49	5.78 (0-74.41)	0.28 (0-1.40)
Extent of algae*	0.599	17.49	0.36	0.57 (0-3)	1.8 (1-2)
Emergent aquatic vegetation 30-60 cm high (%)	0.501	0.05	0.62	14.25 (0-90.50)	9.89 (0-13.58)
Submerged vegetation extent	0.456	0.07	0.40	1.52 (0-4)	2.4 (0-3)

*denotes variables helpful in discriminating between groups.

a Higher PCA loadings indicate variables which contribute more to variation between sites.

b F-to-remove statistics determine the relative importance of variables included in the model\discriminate function; higher values indicate variables most helpful in discriminating between Smelter and Extant sites.

c Tolerance measures the correlation of a variable with other variables in the model and ranges from 0 to 1. Very low tolerances indicate redundant variables, or high correlation with other variables, which may result in an unstable estimate of the discriminate function coefficient.

Table 7. Classification matrices showing the accuracy of case classification by discriminate function analysisa

Classification matrixb			
	Suitable sites	*Unsuitable* sites	% correct
Extant sites	5	0	100
Smelter sites	0	20	100
Total	5	20	100
Jackknifed classification matrixc			
	Suitable sites	*Unsuitable* sites	% correct
Extant sites	4	1	80
Smelter sites	0	20	100
Total	4	21	96

a Each case is classified into the group where the value of its classification function is the largest.

b Represents an optimistic estimate as all cases are used to classify each other case, including that case being classified.

c Uses all cases to classify a particular case, except that case being classified, and so provides a more accurate estimate of the accuracy of case classification.

of shoreline with 'other' types of cover, the percentage of shoreline with terrestrial vegetation less than 5 cm tall and the percentage of bare shoreline, respectively (Table 6). The extent of submerged vegetation and the percentage of shoreline covered by emergent vegetation less than 30 cm tall and 30-60 cm tall were least helpful.

We then used the discriminate function produced by DFA to assign group membership (either Suitable or Unsuitable) to the 11 previously unclassified Smelter sites. Three of these (L53, L54, L55) were classified as Suitable, rather than Unsuitable sites (posterior probability of Suitable membership = 1.00, Table 8). However, examination of the MDS configuration (Fig. 2) and Mahalanobis distances to group centres (Table 8) showed that these sites were comparably distant from both groups.

Discussion

This study suggests that little habitat modification would be required to prepare the smelter wetlands for reintroduction of the Growling Grass Frog. Variation in water quality parameters did not aid discrimination between Smelter and Extant sites, because most Smelter sites fall within the range observed at Extant sites. The Growling Grass Frog tolerates a wide range of water quality conditions. Pyke (2002) recorded adults in water bodies with a pH of 5.6 – 8.4 and electrical conductivity of 101–4800 μS/cm. Parameters at some Extant sites fell outside these ranges. However, the presence of adults in or around acidic, alkaline or saline water bodies does not necessarily mean that the species can breed successfully in such environments. Water quality tests should be repeated

Table 8. Classification of all sites by DFA.

Site	Sites used in initial DFA	Mahalanobis distance-squared from *Smelter/ Unsuitable* group mean[a]	Posterior probabilities for *Smelter/ Unsuitable* membership[b]	Mahalanobis distance-squared from *Extant/ Suitable* group mean[a]	Posterior probabilities for *Extant/ Suitable* membership[b]
$L1_{upper}$		12.4	1.00	47.5	0.00
$L2_{lower}$	●	6.4	1.00	44.4	0.00
UCW	●	8.8	1.00	19.7	0.00
L28		33.4	1.00	92.2	0.00
L29		4.9	1.00	36.4	0.00
L30	●	10.5	1.00	35.3	0.00
L31		20.6	1.00	72.2	0.00
L32	●	4.0	1.00	20.5	0.00
L33	●	8.9	1.00	47.9	0.00
L35	●	4.1	0.97	11.4	0.03
L42	●	2.9	1.00	26.6	0.00
L43		10.9	1.00	58.2	0.00
L45	●	7.5	1.00	36.5	0.00
L52	●	8.7	1.00	31.4	0.00
L53		921.9	0.00	892.2	1.00
L54		86854.9	0.00	86708.5	1.00
L55		14928.4	0.00	14814.4	1.00
L56		29.3	0.80	32.0	0.20
$L57_{upper}$		29.4	1.00	57.3	0.00
$L57_{lower}$		41.5	1.00	102.1	0.00
Bridgewater Lakes	●	18.3	0.00	3.0	1.00
Swan Lake	●	25.6	0.00	6.1	1.00
Lake Monibeong	●	33.9	0.00	2.2	1.00
Lake Pertobe	●	24.0	0.00	1.2	1.00
Tower Hill	●	37.9	0.00	9.6	1.00

a The smaller the Mahalanobis distance-squared values, the closer, or more similar, a site is to the mean of each group.

b Posterior probabilities for group membership represent the likelihood of each site actually belonging to either the Smelter or Extant groups after classifying each site using the discriminate function (prior probability of all sites for group membership = 0.500 for each group).

before reintroduction due to the dynamic nature of these parameters and the potential impacts of poor water quality on tadpole growth, development and survival (e.g. Cummins 1986; Freda and Dunson 1986; Ferraro and Burgin 1993; Christy and Dickman 2002).

Wetland size also did not aid discrimination between Smelter and Extant sites. All known Extant populations in the region were found at large, permanent water bodies, so the influence of wetland size on Growling Grass Frog populations is worth considering. The persistence of Growling Grass Frogs at these larger lakes may be due to the year-round presence of water and relatively little anthropogenic disturbance. Permanent wetlands are advantageous to this species because it breeds during the warmer months of the year (Pyke 2002), when many smaller water bodies may be dry. Wetlands with recent records of Growling Grass Frogs are located within parks and reserves, and although used for human recreation, these wetlands may have experienced less disturbance and modification than those on private land. Populations at smaller wetlands may also be at greater risk of local extinction if there are no suitable wetlands nearby or if barriers, such as roads or urban areas, prevent individuals from reaching other patches of suitable habitat (Vos and Chardon 1998; Knutson et al. 1999; Marsh et al. 1999; Findlay and Bourdages 2000; Trombulak and Frissell 2000; Carr and Fahrig 2001). However, the smelter wetlands form a close network of apparently suitable water bodies, where the species previously occurred prior to construction of the smelter (Alcoa and Kinhill 1980).

Four of the measured habitat parameters discriminated between Extant and Smelter sites: the extent of algae, the percentage of shoreline covered by terrestrial vegetation less than 5 cm tall, the percentage of shoreline covered by "other" cover, and by bare ground. We consider that the first two parameters would be unlikely to affect the

re-establishment of the Growling Grass Frog in the smelter wetlands (see below). By contrast, we recommend that the latter two parameters should be addressed prior to reintroduction of the species, in addition to other potential issues. Smelter wetlands with the smallest Mahalanobis distances from Extant group centres (UCW, L32, L35 and L42 – see Table 8) are likely to be most suitable for the Growling Grass Frog, and reintroduction efforts should be initially concentrated on these sites.

The lesser extent of algae recorded in the smelter wetlands is considered unlikely to limit the Growling Grass Frog in the smelter wetlands. While the extensive areas of algae at Extant sites may provide an important food source for tadpoles of Growling Grass Frogs and other species, as well as a foraging substrate for adults (G. Heard, pers. comm. 2006), tadpoles consume other plant matter and detritus (Anstis 2000) and adults forage on other substrates (pers. obs.). In addition, the measure of algae primarily consisted of floating mats of filamentous algae, rather than algae growing on submerged plants, rocks and other material, which Growling Grass Frogs tadpoles are likely to consume (P. Robertson, pers. comm. 2003, Wildlife Profiles Pty Ltd). The presence of numerous tadpoles and frogs of other species in and around the smelter wetlands (pers. obs.) indicated that food for tadpoles is abundant in the area.

The high proportion of "other" cover types along Extant shorelines may provide Growling Grass Frogs with important diurnal and winter shelter sites, as well as foraging opportunities and refuge from predators. Although a lesser extent of such cover was recorded at Smelter sites, potential shelter sites were recorded near most smelter wetlands. Therefore, this parameter is also not expected to hinder reintroduction efforts.

The remaining habitat parameters that differed between Extant and Smelter sites should be addressed before reintroduction of the species is attempted at the smelter. Smelter wetlands had more short terrestrial vegetation and bare ground around their edges than Extant sites and, therefore, proportionally less emergent aquatic vegetation, an important habitat component for Growling Grass Frogs. Robertson et al. (2002) found that the likelihood of a water body being occupied by Growling Grass Frogs increased with the extent of emergent vegetation, and adults have often been observed basking amongst such vegetation (Osbourne et al. 1996; Tyler 1997), which also provides a refuge from predators. The deficiency in emergent aquatic vegetation at some Smelter wetlands could be remedied by planting emergent macrophytes such Eleocharis spp. Areas of bare ground and short terrestrial vegetation should be retained, however, as adults often forage in these more open areas (Skye Wassens, pers. comm. 2006; pers. obs.; Heard 2008), which may provide ideal vantage points to spot and ambush prey.

In addition to the suitability of structural habitat and water quality, a number of other factors may reduce the likelihood of successful re-establishment of Growling Grass Frogs at the smelter wetlands and elsewhere, and these should be considered prior to any reintroduction efforts. While there have been no documented attempts to reintroduce or translocate Growling Grass Frogs, a number of issues have been encountered when trying to reintroduce populations of the closely related Green and Golden Bell Frog L. aurea in NSW (Daly et al. 2008; Pyke et al. 2008). To date, these introductions have had limited success. These issues include, but are not necessarily limited to, the presence of the chytrid fungus Bactrachochytrium dendrobatiodis pathogen within existing frog populations or animals to be released; presence of introduced terrestrial and aquatic predators (e.g. foxes and cats, trout and Mosquito Fish); and security of the proposed reintroduction site.

Chytrid has been implicated in the declines of many amphibian species in Australia and, while some species may appear healthy while infected with the fungus (e.g. L. verreauxii, as described in Daly et al. 2008), Green and Golden Bell Frogs are apparently highly susceptible (Daly et al. 2008). Growling Grass Frogs are also likely to be susceptible to chytrid and, prior to release of individuals at a site, samples should be collected from existing frog populations to establish whether chytrid is present. If so, reintroduction of the species should be reconsidered. Screening of animals scheduled for release should occur in order to prevent the infection of chytrid-free populations. This will also limit potential confounding factors when trying to determine reasons for an unsuccessful release.

Daly et al. (2008) noted that, in addition to introduced species, potential predators also include native species (e.g. snakes, turtles, wading birds and raptors). Pyke et al. (2008) found that the presence of any fish, either native or exotic, reduced the survival of tadpoles to metamorphosis. While it has been established that Mosquito Fish prey on frog eggs and tadpoles (Morgan and Buttemer 1996; Pyke and White 2000), no Green and Golden Bell Frog tadpoles or metamorphs were recorded in or around ponds containing only native fish in the 12 months following the release of tadpoles (Pyke et al. 2008).

Prior to reintroduction, control programs for (at least) foxes and cats should be in place. It may be more difficult to limit the effects of predation by native species with similar control programs and methods that physically exclude predators may be more appropriate. The observations of Pyke et al. (2008) suggest that comprehensive fish surveys should be undertaken prior to releasing tadpoles, or juvenile or adult frogs, and any fish detected should be removed from potential reintroduction sites. Depending on the hydrology of the site (e.g. water source, connectivity with other wetlands) and available control methods (e.g. manual removal or poisons such as Rotenone), complete eradication of fish populations may prove to be problematic.

The object of reintroduction programs should be to establish free-ranging, self-sustaining, viable populations that require only limited long-term management (Lindenmayer 1994). Investigations of habitat suitability prior to reintroduction are important to ensure that sufficient suitable habitat exists at release sites, because the presence of high quality habitat can greatly increase the likelihood of success (Griffith et al. 1989) and because habitat destruction, modification and fragmentation are

major factors in the decline of many species (Lindemayer 1994). In cases such as the present study, when relatively little is known about the species' habitat requirements, valuable information can be acquired from reintroduction efforts, particularly regarding conditions required for breeding. For example, if Growling Grass Frog tadpoles were introduced to a number of smelter wetlands that varied in, say, salinity, the extent of emergent vegetation, or the presence of fish, the outcome at each wetland (i.e. tadpole survival, metamorphosis and whether subsequent breeding occurs) can provide valuable information regarding the species' habitat requirements. In order to draw causal links between habitat variables and the success or otherwise of reintroductions, it is imperative that such reintroductions have a rigorous experimental design, including adequate replication. Post-release surveys of tadpoles and frogs (e.g. presence and distribution of individuals, patterns of habitat use) will also be critical in determining whether reintroductions have been successful. Information gained from an experimental reintroduction at the smelter could aid in the design and planning of Growling Grass Frog reintroductions at other suitable sites, and guide recovery efforts and habitat management decisions at extant sites.

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References

Adams, M.J. 1999. Correlated factors in amphibian decline: exotic species and habitat change in western Washington. *Journal of Wildlife Management* **63:** 1162-1171.

Alcoa and Kinhill 1980. Alcoa Portland Aluminium Smelter: Environmental Effects Statement. Alcoa of Australia and Kinhill Planners: Melbourne, Victoria.

Anstis, M. 2002. *Tadpoles of South-eastern Australia: a Guide with Keys*. New Holland Publishers, Frenchs Forest.

Boulton, I.C., Cooke, J.A. and Johnson, M.S. 1994. Experimental fluoride accumulation and toxicity in the short-tailed field vole (*Microtus agrestis*). *Journal of the Zoological Society of London* **234:** 409-421.

Brake, L., Clark, I., Clay, R. and Savarton, F. 1991. The Master Plan for the Smelter in the Park. Centre for Environmental and Recreation Management, University of South Australia.

Carr, L.W. and Fahrig, L. 2001. Effect of road traffic on two amphibian species of differing vagility. *Conservation Biology* **15:** 1071-1078.

Christy, M.T. and Dickman, C.R. 2002. Effects of salinity on tadpoles of the green and golden bell frog (*Litoria aurea*). *Amphibian-Reptilia* **23:** 1-11.

Clay, R., Clark, I. and Brake, L. 1994. A report on The Wetlands Workshop held at The Smelter in the Park, 8-10 December 1993.

Cogger, H.G. 2000. *Reptiles and Amphibians of Australia.* 6th edition. Reed Books, Melbourne.

Coulson, G., Hill, J., McKenzie, J. and Walters, B. 2000. The Smelter in the Park: managing wildlife for biodiversity. Pp. 360-371 in Nature Conservation 5: Nature Conservation in *Production Environments: Managing the Matrix*, edited by J.L. Craig, N. Mitchell, and D.A. Saunders. Surrey Beatty and Sons, Chipping Norton.

Cummins, C.P. 1986. Effects of aluminium and low pH on growth and development in *Rana temporaria* tadpoles. *Oecologia* **69:** 248-252.

Daly, G., Johnson, P., Malolakis, G., Hyatt, A. and Pietsch, R. 2008. Reintroduction of Green and Golden Bell Frog *Litoria aurea* to Pambula on the south coast of New South Wales. *Australian Zoologist* **34:** 261-270.

Denton, J.S., Hitchings, S.P., Beebee, T.J.C. and Gent, A. 1997. A recovery program for the natterjack toad (Bufo calamita) in Britain. *Conservation Biology* **11:** 1329-1338.

Draper, R. 2001. The Growling Grass Frog Project (Warty Bell Frog) needs your help. Land for Wildlife News: 4(9), Department of Natural Resources and Environment: Victoria.

Ferraro T.J. and Burgin, S. 1993. Review of environmental factors influencing the decline of Australian frogs. Pp. 205-216 in *Herpetology in Australia*. A Diverse Discipline, edited by D. Lunney and D. Ayers. Royal Zoological Society of NSW, Sydney.

Findlay, C.S.T. and Bourdages, J. 2000. Response time of wetland biodiversity to road construction on adjacent lands. *Conservation Biology* **14:** 86-94.

Freda, J. and Dunson, W.A. 1986. Effects of low pH and other chemical variables on the local distribution of amphibians. *Copeia* 1986(2): 454-466.

Griffith, B., Scott, J.M., Carpenter, J.W. and Reed, C. 1989. Translocation as a species conservation tool: status and strategy. *Science* **245:** 477-480.

Hamer, A.J., Lane, S.J. and Mahony, M.J. 2002. Management of freshwater wetlands for the endangered green and golden bell frog (*Litoria aurea*): roles of habitat determinants and space. Biological Conservation 106: 413-424.

Heard, G.W., Robertson, P. and Scroggie, M. P. 2008. Microhabitat preferences of the endangered Growling Grass Frog *Litoria raniformis* in southern Victoria. *Australian Zoologist* **34:** 414-425.

Hero, J-M., Littlejohn, M. and Marantelli, G. 1991. Frogwatch Field Guide to Victorian Frogs. Wetlands Conservation Program, Department of Conservation and Environment: Victoria.

Hero, J-M., Morrison, C., Gillespie, G., Roberts, J.D., Newell, D., Meyer, E., McDonald, K., Lemckert, F., Mahony, M., Osborne, W., Hines, H., Richards, S., Hoskins, C., Clarke, J., Doak, N. and Shoo, L. 2006. Overview of the conservation status of Australian frogs. *Pacific Conservation Biology* **12:** 313-320.

Hill, J., Brake, L. and Clark, I. 2000. The Smelter in the Park: using environmental care to improve business outcomes. Pp. 345-359 in *Nature Conservation 5: Nature Conservation in Production Environments: Managing the Matrix*, edited by J.L. Craig, N. Mitchell, and D.A. Saunders. Surrey Beatty and Sons, Sydney.

Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyers, A.H. and Kuzmin, S.L. 2000. Quantitative evidence for global amphibian declines. *Nature* **404:** 752-755.

James, F.C. and McCulloch, C.E. 1990. Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annual Review of Ecology and Systematics* **21:** 129-66.

Kierdorf, U. and Kierdorf, H. 2000. Comparative analysis of dental fluorosis in roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*): interdental variation and species differences. *Journal of the Zoological Society of London* **250:** 87-93.

Kiesecker, J.M., Blaustein, A.R. and Belden, L.K. 2001. Complex causes of amphibian population declines. Nature **410:** 681-683.

Knapp, R.A. and Matthews, K.R. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conservation Biology* **14:** 428-438.

Knutson, M.G., Sauer, J.R., Olsen, D.A., Mossman, M.J., Hemesath, L.M. and Lannoo, M.J. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. *Conservation Biology* **13:** 1437-1446.

Lindenmayer, D.B. 1994. Some ecological considerations and computer-based approaches for the identification of potentially suitable release sites for reintroduction programmes. Pp. 1-5 in *Reintroduction Biology of Australian and New Zealand Fauna*, edited by M. Serena. Surrey Beatty and Sons, Chipping Norton.

Letnic, M. and Fox, B.J. 1997. The impact of industrial fluoride fallout on faunal succession following sand mining of dry sclerophyll forest at Tomago, NSW, II. Myobatrachid frog recolonization. *Biological Conservation* **82:** 137-46.

Madden, K.E. and Fox, B.J. 1997. Arthropods as indicators of the effects of fluoride pollution on the succession following sand mining. *Journal of Applied Ecology* **34:** 1239-1256.

Mahoney, M. 1999. Review of the declines and disappearances within the bell frog species group (*Litoria aurea* species group) in Australia. Pp. 81-93 in *Declines and Disappearances of Australian Frogs*, edited by A. Campbell. Environment Australia, Canberra.

Marsh, D.M. and Pearman, P.B. 1997. Effects of habitat fragmentation on the abundance of two species of leptodactylid frogs in an Andean montane forest. *Conservation Biology* **11:** 1323-1328.

Marsh, D.M., Fegraus, E.C. and Harrison, S. 1999. Effects of breeding pond isolation on the spatial and temporal dynamics of pond use by the tungara frog, *Physalaemus pustulosus*. *Journal of Animal Ecology* **68:** 804.

Morgan, L.A. and Buttemer, W.A. 1996. Predation by the non-native *Gambusia holbrooki* on small *Litoria aurea* and *L. dentata* tadpoles. *Australian Journal of Zoology* **30:** 143-149.

DNRE 2002. Atlas of Victorian Wildlife. Department of Natural Resources and Environment: Victoria.

Osborne, W.S., Littlejohn, M.J. and Thomson, S.A. 1996. Former distribution and apparent disappearance of the *Litoria aurea* complex from the Southern Tablelands of New South Wales and the Australian Capital Territory. *Australian Journal of Zoology* **30:** 190-198.

Pounds, J.A., Fogden, M.P., Savage, J.M. and Gorman, G.C. 1997. Tests of null models for amphibian declines on a tropical mountain. *Conservation Biology* **11:** 1307-1322.

Pyke, G.H. 2002. A review of the biology of the Southern Bell Frog *Litoria raniformis* (Anura: Hylidae). *Australian Zoologist* **32:** 32-48.

Pyke, G.H. and White, A.W. 1996. Habitat requirements for the Green and Golden Bell Frog *Litoria aurea* (Anura: Hylidae). *Australian Zoologist* **30:** 224-232.

Pyke, G.H. and White, A.W. 2000. Factors influencing predation on eggs and tadpoles of the endangered green and golden bell frog *Litoria aurea* by the introduced plague minnow *Gambusia holbrooki*. *Australian Zoologist* **31:** 12-31.

Pyke, G.H. and White, A.W. 2001. A review of the biology of the Green and Golden Bell Frog *Litoria aurea. Australian Zoologist* **31:** 563-598.

Pyke, G.H., Rowley, J., Shoulder, J. and White, A.W. 2008. Attempted Introduction of the endangered Green and Golden Bell Frog to Long Reef Golf Course: A step towards recovery? *Australian Zoologist,* **34:** 361-372.

Quinn, G.P. and Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Robertson, P., Heard, G. and Scroggie, M. 2002. The ecology and conservation status of the Growling Grass Frog (*Litoria raniformis*) within the Merri Creek corridor. Interim report to the Department of Natural Resources and Environment: Victoria.

Romanowski, N. 1998. *Aquatic and Wetland Plants: a Field Guide for Non-tropical Australia*. UNSW Press, Sydney.

Rounsevel, D. and Swain, R. 1993. Current issues in the conservation of the terrestrial herpetofauna of Tasmania. Pp. 71-74 in *Herpetology in Australia*. A Diverse Discipline, edited by D. Lunney and D. Ayers, Royal Zoological Society of NSW, Sydney.

SAC 2000. Final recommendation on a nomination for listing: *Litoria raniformis* Kerferstein 1867 Warty Bell Frog (Nomination Number 486 (316)). Flora and Fauna Guarantee, Scientific Advisory Committee. Department of Natural Resources and Environment: Melbourne.

Sainty, G.R. and Jacobs, S.W.L. 1994. *Waterplants in Australia*. Third edition, Sainty and Associates, Darlinghurst.

Soderquist, T.R. 1994. The importance of hypothesis testing in reintroduction biology: examples from the reintroduction of the carnivorous marsupial *Phascogale tapoatafa*. Pp. 159-64 in *Reintroduction Biology of Australian and New Zealand Fauna,* edited by M. Serena. Surrey Beatty and Sons, Chipping Norton.

Sutherland, W.J. 2000. *The Conservation Handbook: Research, Management and Policy*. Blackwell Science, Oxford.

Thomson, S.A., Littlejohn, M.J., Robinson, W.A. and Osborne, W.S. 1996. Taxonomy of the *Litoria aurea* complex: a re-evaluation of the Southern Tableland populations of the Australian Capital Territory and New South Wales. *Australian Zoologist* **30:** 158-169.

Trombulak, S.C. and Frissell, C.A. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation Biology* **14:** 18-30.

Tyler, T.J., Liss, W.J., Hoffman, R.L. and Ganio, L.M. 1998. Experimental analysis of trout effects on survival, growth and habitat use of two species of ambystomatid salamanders. *Journal of Herpetology* **32(3):** 345-349.

Tyler, M.J. 1997. The Action Plan for Australian Frogs. *Wildlife Australia*, Canberra.

Vos, C.C and Chardon, J.P. 1998. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *Journal of Applied Ecology* **35:** 44-56.

Webb, C. and Joss, J. 1997. Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Australian Zoologist* **30:** 316-324.